(54) Title: METHOD OF ISOLATING AND PURIFYING A BIOMACROMOLECULE

(57) Abstract

The invention provides a method of separating a biomacromolecule which comprises the steps of providing a separation system including a filter element which comprises a composite filtration medium, said composite filtration medium comprising a filtration layer on the upstream surface of which are located insoluble stationary phase particulates, the particulates being capable of binding to a biomacromolecule or class of biomacromolecules, a reservoir containing a solution mixture comprising at least one biomacromolecule as solute, and a pump and associated tubing to form a closed loop assembly, and recirculation pumping the solution mixture through the filter cartridge so as to bind the at least one biomacromolecule to the stationary phase particulate so as to form a biomacromolecule:stationary phase particulate product. An eluting solution can be pumped through the closed loop assembly which is capable of reversing the biomacromolecule:stationary phase particulate product binding interaction so as to liberate the biomacromolecule.

5                    METHOD OF ISOLATING AND PURIFYING
                            A BIOMACROMOLECULE

     Field of the Invention
          This invention relates to a method for the
10   separation and purification of a biomacromolecule from
     a solution which comprises one or a plurality of
     biomacromolecules, especially on a large scale.  The
     purified biomacromolecules are useful therapeutic or
     diagnostic agents.
15

     Background of the Invention
          Biomacromolecules are constituents or products of
     living cells and include proteins, carbohydrates,
     lipids, and nucleic acids.  Detection and
20   quantification as well as isolation and purification of
     these materials have long been objectives of
     investigators.  Detection and quantification are
     important diagnostically, for example, as indicators of
     various physiological conditions such as diseases.
25   Isolation and purification of biomacromolecules are
     important for therapeutic purposes such as when
     administered to patients having a deficiency in the
     particular biomacromolecule, when utilized as a
     biocompatible carrier of some medicament, and in
30   biomedical research.  Biomacromolecules such as enzymes
     which are a special class of proteins capable of
     catalyzing chemical reactions are also useful
     industrially; enzymes have been isolated, purified, and
     then utilized for the production of sweeteners,
35   antibiotics, and a variety of organic compounds such as
     ethanol, acetic acid, lysine, aspartic acid, and
     biologically useful products such as antibodies and
     steroids.

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In their native state _in vivo_, structures and
corresponding biological activities of these
biomacromolecules are maintained generally within
fairly narrow ranges of pH and ionic strength.
5 Consequently, any separation and purification operation
must take such factors into account in order for the
resultant, processed biomacromolecule to have potency.

Chromatography is a separation and purification
operation that is often performed on biological product
10 mixtures. It is a technique based on the interchange
of a solute between a moving phase, which can be a gas
or liquid, and a stationary phase. Separation of
various solutes of the solution mixture is accomplished
because of varying binding interactions of each solute
15 with the stationary phase; stronger binding
interactions generally result in longer retention times
when subjected to the de-binding effects of a mobile
phase compared to solutes which interact less strongly
and, in this fashion, separation and purification can
20 be effected.

Efforts to utilize polydisperse particles as
stationary phases for separating biomacromolecules by
incorporation within a porous fiber matrix are
disclosed in U.S. Patent Nos. 4,384,957 and 4,488,969.
25 Resultant composite sheet structures were cut into
circular shapes and stacked to form columns.

Liquid cartridge filters have been developed over
the years which represent a highly efficient format for
the interaction of a liquid stream and a solid matrix.
30 Furthermore, these filters operate at relatively high
flow rates, e.g., liters per minute, and at relatively
low pressures.

In tangential flow or radial membrane cartridge
filters, the filtering element is presented in a plane
35 parallel to the liquid stream flow, and two effluents
or permeates are produced, one filtered or processed by

passing through the filtering element and another not. While these filter arrangements operate at low pressures and the unprocessed permeate can in theory be recycled, these systems are intrinsically more

5  complicated and slower to completely process a liquid stream because of relatively low flow through the element; also, if filtering elements were modified in some fashion to retain biomacromolecules, complete retention would be required in one pass through the

10  element.

In "dead end" filters the filtering element is presented perpendicularly to the direction of flow of the liquid stream. All the liquid stream is required to pass through the element and only one permeate is

15  produced. Considered as a separation unit in which separation is occurring by interaction with a stationary phase on or within the filtering element, the dead end cartridge filter would be analogous to a very wide, but shallow column. At high flow rates

20  single pass retention of the biomacromolecule may be relatively low but by repeatedly cycling the effluent high percentages of the biomacromolecule can be retained.

Bioseparations have been conducted using modified

25  filter cartridges. U.S. Patent No. 5,155,144 discloses microporous sheets comprising modified polysaccharide particulates such as diethylaminoethyl cellulose, a typical ion exchange chromatography stationary phase, dispersed within a polymeric medium. It is suggested

30  that these sheets can further be configured into a dead end filter cartridge. Employing recirculation of effluent, a lead ion treated resin was evaluated as a shallow column between two stainless steel grids for the analytical separation of D-xylose (cf. A.M. Wilhelm

35  and J.P. Riba, J. Chromatog., 1989, 484, 211-223). The resulting packed bed reactor system was evaluated to

determine hydrodynamic conditions for particles for
ultimate employment in columns for production liquid
chromatography at relatively high system pressures and
low flow rates.

5       U.S. Patent No. 4,774,004 discloses the use of
cartridge filters that are "charged" with filtration
aids which are layered silicates that essentially
function as ion exchange media and, optionally,
kieselguhr or activated carbon.  The resultant

10  structures were useful for removing surfactants and
"dissolved soil" from dry cleaning solvents.  Details
of the charging procedure, quantities of filtration
aids employed, recycle flow rates, and operating system
pressures are scant.  No separation of one or more

15  biomacromolecules was conducted nor contemplated.


## Summary of the Invention

        Briefly, this invention provides a method of
separating (can include purifying) a biomacromolecule

20  comprising the steps of (a) providing a separation
system containing a dead end filter cartridge
comprising a composite filter medium on the upstream
surface of which are located stationary phase
particulates capable of binding with a

25  biomacromolecule, a reservoir containing a solution
comprising at least one biomacromolecule as solute, and
a pump and associated tubing to form a closed loop
assembly, (b) recirculation pumping the solution
through the filter cartridge so as to selectively bind

30  one biomacromolecule or more than one biomacromolecule
in the case of related biomacromolecules to the
stationary phase particulate so as to form a
biomacromolecule:stationary phase particulate product,
and (c) optionally, pumping an eluting solution through

35  the closed loop assembly which is capable of reversing
the biomacromolecule:stationary phase particulate

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product binding interaction so as to liberate the
biomacromolecule.

For use in this method, this invention provides a
filter element comprising a composite filtration

5   medium, the composite filtration medium comprising a
filtration layer on the upstream surface of which are
located insoluble stationary phase particulates, the
particulates being capable of binding to a
biomacromolecule.

10   In another aspect, there is provided a filter
cartridge including the above-described filter element.

In yet another aspect, there is provided a
separation filter assembly comprising the filter
cartridge and a filter cartridge housing, the

15  stationary phase particulate of the composite
filtration medium of the invention being capable of
binding a biomacromolecule.

This invention provides a method for separating,
purifying, or concentrating a biomacromolecule solute

20  from a solution containing other biomacromolecular
solute compounds.  The method is conducted at
relatively low pressure and is especially suitable for
large scale bioseparations.

More particularly, the method of the invention

25  provides a liquid filter cartridge comprising a
composite filter medium contained within a suitable
housing which is connected to a pump and a solution
reservoir.  The novel composite filter medium is
prepared by a process in which a slurry comprising at

30  least one of chromatographic adsorption, ion exchange,
affinity, and hydrophobic stationary phase particulates
in a liquid (generally water) is pumped through to
partially load a filtration layer such that the
stationary phase particulates are principally located

35  on the upstream surface of the filtration layer.  A
solution of a biological mixture to be separated is

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then pumped through the filter cartridge in order for
the biological solute of interest to be separated from
the solution by a binding association with the
stationary phase.  The resultant solution with the
5  selected biomacromolecular solute removed (or its
concentration decreased) may be utilized.  The
procedure is more commonly performed, however, to
recover the separated biological solute.  During
elution or an isolation step, a solution which can
10  effect reversal of the binding to the stationary phase
is next pumped through the filter cartridge, preferably
in a volume of solution smaller than the initial volume
of the biological solution mixture.  Binding of the
selected biomacromolecule solute from a solution which
15  passes through the filter element can be by sorption or
chemical reaction.  Preferred binding mechanisms
include adsorption, ion exchange, hydrophobic
association, and affinity binding.  In a separate step,
the binding can be reversed so as to isolate and purify
20  the previously bound biomacromolecule.

   In this application:

   "biomacromolecule" means a component or product of
a cell such as a protein, carbohydrate, lipid, or
nucleic acid, possessing a molecular weight of at least
25  500;

   "filtration layer" means a sheet-like woven or
nonwoven porous material which can comprise one or more
individual layers which can be combined to provide a
single sheet; the average pore size is greater than 1
30  micrometer and up to 50 micrometers;

   "composite filtration medium" means a filtration
layer comprising stationary phase particulates located
on the upstream surface thereof; the medium can sustain
a flux rate of at least 0.01 cm/min at a filter
35  cartridge pressure of at most 0.25 MegaPascals (MPa);

"filter element" or "filtering element" or
"filtration element" means a composite filtration
medium configured for fluid passage; it is the actual
component of a separation filter assembly which
5  accomplishes the filtering/separating/purifying
operation;

"filter cartridge" means a dead end filtering
device which is preferably cylindrical in shape;

"filter cartridge housing" means a support
10  structure for a filter cartridge;

"separation filter assembly" means a housing
containing a dead end filter cartridge comprising a
composite filter medium on the upstream surface of
which are located stationary phase particulates;

15        "separation system" means a solution mixture
comprising at least one biomacromolecular solute
contained in a reservoir, a separation filter assembly,
a pump, and associated tubing;

"flux rate" means the velocity of a liquid stream
20  passing through a filtering element and is equal to
flow rate divided by the surface area of the filtration
layer.  Described in this way, flow of a liquid stream
can be characterized and is independent of the size of
the filtration layer.  Flux rate also contributes to
25  pressure drop across a filter, i.e., increased flux
rates generally mean increased system pressures.  In
commercial filter cartridge applications, it is highly
desirable to provide a filter of minimum size which
will process a maximum amount of liquid stream.
30  Therefore, it is desirable that flux rate be increased
by increasing the flow rate;

"stationary phase particulates" mean insoluble
particulates that can form a binding association with a
biomacromolecular component of interest in a solution
35  mixture. Specific binding associations include:

adsorption, ion exchange, hydrophobic, and affinity
interactions;

"cryo-poor" means blood plasma from which
undissolved solids have been removed after a
5 freeze/thaw cycle;

"insoluble" means not more than 1 part
particulates dissolves in 100 parts of solvent at 23°C;
and

"filter cartridge pressure" means the difference
10 between inlet, or upstream, and outlet, or downstream,
pressures across the filter cartridge unit in a
separation system.

The present invention process overcomes problems
of prior art cartridge filters employed for the
15 separation of biomacromolecules. Prior art filters
containing stationary phase particulates within a
filtering element present manufacturing challenges and
offer only limited capacities. The hazards of air-
borne particulates are well known and related
20 manufacturing problems can easily arise in construction
of filter cartridges which comprise small stationary
phase particulates entrapped within a filter element.
Prior art systems are also capacity limited in that
more particulates must be loaded into a filtering
25 element in order to increase the quantity of
biomacromolecule separated. Higher loading of
particulates gives rise to a filtering element with
reduced porosity and concomitant increased operating
system pressures.
30     The present invention overcomes these problems of
prior art filters by significantly reducing handling of
dry stationary phase particulates and by providing high
loading capacity of particulates at relatively low
filter cartridge pressures.
35

## Brief Description of the Drawing

FIG. 1 is a schematic illustration of a cross-
section of a composite filtration medium of the
invention;

5       FIG. 2 is a perspective view of the embossed
pattern on a composite filtration medium of the
invention;

FIG. 3 is a perspective view of a cylindrically
pleated filter element of the invention;

10      FIG. 4 is a perspective view of support members
for a cylindrical filter cartridge of the invention;

FIG. 5 is a perspective view of a separation
filter assembly of the invention;

FIG. 6 is a schematic illustration of a separation
15  system of the invention;

FIG. 7 depicts stained protein gel electrophoresis
patterns of comparative proteins and a purified protein
of the invention.


## 20 Detailed Description of the Drawing

FIG. 1 is a schematic illustration of a cross-
section of a composite filtration medium 10 comprising
a preferred nonwoven web as surface filtration layer 11
which can be one or more individual layers, upon the
25  upstream surface of which are located insoluble
stationary phase particulates 12. The nonwoven
filtration layer 11 which possesses uniform porosity
and well-defined pores can comprise coarse upstream
prefilter layer 13, filtration layers 14 comprising a
30  multiplicity of nonwoven filtration layers having
increasingly finer downstream porosity, and a
downstream nonwoven cover layer 15.

FIG. 2 is an illustration of a preferred
embodiment of the invention. There is shown a
35  perspective view of a nonpleated portion of a pattern
of embossed shapes 22 on composite filtration medium 20

utilized to produce filter cartridges. Embossing is
conducted to increase frontal surface area and more
completely define the surface filtering element. The
insoluble stationary phase particulates are omitted

5    from the illustration for clarity.

FIG. 3 is a perspective view of a longitudinally
extended cylindrically pleated filter element 30 of a
preferred embodiment of the invention; radial pleats 32
of preferred compound radially pleated filtration

10   element 30 of the invention are shown; again,
stationary phase particulates are omitted for clarity.

FIG. 4 is a perspective view which illustrates
inner and outer supplemental support members for
cylindrical filter cartridge 40, which is a preferred

15   embodiment of the invention. External support
structure 41, such as a scrim or screen with a
multiplicity of holes, can provide additional support
in an inward-out fluid flow mode to reduce the
likelihood of rupturing the filter element. Similarly,

20   inner support structure 42 consisting of a scrim or
screen, or a porous casing or similar construction can
provide support to prevent the filter element (not
shown) from collapsing under high pressure applications
in a preferred outward-in fluid flow situation. In

25   both cases, the supplemental support structures are
normally attached to endpieces 43 of the filter
cartridge to provide an integral unit.

FIG. 5 is a perspective view of a separation
filter assembly 70 of the invention, this being a

30   preferred embodiment of the invention. Filter housing
71 contains a filter cartridge (not shown). In the
separation loop, inlet port 72 allows the solution
mixture to enter the filter cartridge in the preferred
outward-in mode. The liquid exits separation filter

35   assembly 70 through outlet port 73. In a preferred
assembly, the separation head 74 is attached to filter

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housing 71 by a mechanical clamp 75 employing a
threaded bolt (not shown) with tension adjusting
control knob 76.  In the isolation loop, inlet port 77
allows de-binding solution to enter the filter
5  cartridge in the preferred outward-in mode, and the
resultant solution now containing the desired
biomacromolecule solute exits separation filter
assembly 70 through outlet port 78.

FIG. 6 is a schematic illustration of a separation
10  system 80 of the invention.  Reservoir 81 contains
aqueous stationary phase particulate slurry 82 and/or
biomacromolecule solution mixture 82, with stirring
being provided by stirring apparatus 83.  Slurry or
solution 82 is pumped from outlet tube 84 by pump 85
15  through separation filter assembly 86 (which contains
stationary phase particulates located on the upstream
surface of the filtration layer of a filter cartridge
(not shown)) and back into the reservoir via inlet tube
87 (arrows show direction of liquid flow).

20      FIG. 7 shows a stained polyacrylamide
electrophoretic gel 90.  Lane A depicts the protein
electrophoresis separation pattern achieved with a
commercial mixture (available from Pharmacia LKB
Biotechnology, Uppsala, Sweden) of known proteins
25  (listed in order of increasing migration distance from
the origin; approximate molecular weight in Daltons is
also given):  phosphorylase b (94,000), bovine serum
albumin (67,000), ovalbumin (43,000), carbonic
anhydrase (30,000), soybean trypsin inhibitor (20,100),
30  and alpha-lactalbumin (14,400).  Lane B depicts the
large number of proteins comprising cryo-poor human
blood plasma.  Lane C depicts the protein
electrophoresis pattern of commercial (Sigma Chemical
Co., St. Louis, MO) human immunoglobulins, and lane D
35  depicts the protein electrophoresis pattern of the
protein eluate of Example 5.

### Detailed Description of Preferred Embodiments

This invention provides a method of isolating and purifying a biomacromolecule comprising a separation filter assembly including a filter element which
5 incorporates stationary phase particulates which can bind to a biomacromolecule on the upstream surface of a filtration layer. The separation filter assembly comprises a liquid filter cartridge which includes the above-described filter element and a suitable cartridge
10 housing for the filter element connected to a reservoir of solution comprising one, or preferably two or more biomacromolecules. The filter cartridge is connected by suitable tubing to a pump capable of passing the solution, which can include a selected biomacromolecule
15 to be separated by binding to particulate or an eluting solution to release the bound biomacromolecule, through the filter element and back into the reservoir so that the resultant solution can be repeatedly cycled through the filter element for further capture of the free
20 biomacromolecule to complete the separation, or, if desired, to elute the bound biomacromolecule.

More particularly, the invention provides a method of separating or purifying a biomacromolecule, the method comprising the steps of:
25     1)     providing a separation system containing a dead end filter cartridge comprising a composite filter medium, on the upstream surface of which are located stationary phase particulates capable of adsorption, ion exchange, hydrophobic, or affinity binding with a
30 biomacromolecule, a reservoir containing a solution mixture comprising one or more than one biomacromolecule solute, a pump and associated tubing to form a closed loop system;

2)    recirculation pumping the solution mixture
through the filter cartridge assembly to accomplish
binding of the selected biomacromolecule to the
stationary phase particulates, the pumping through the
5 filter element being conducted with a flux rate of at
least 0.01 cm/minute, preferably at least 0.10
cm/minute, and more preferably at least 0.30 cm/minute
at a filter cartridge pressure of at most 0.25 MPa;
3)    optionally, washing the biomacromolecule:
10 stationary phase particulate product with suitable
liquid to remove unwanted biomacromolecules and other
solutes not bound to the stationary phase particulates
by the selected adsorption, ion exchange, hydrophobic
or affinity binding interaction, in an open loop or one
15 pass procedure; and
4)    optionally pumping, preferably a decreased
volume (compared to the original solution mixture
volume), of a debinding solution containing a solute
which will reverse the biomacromolecule:stationary
20 phase particulate binding interaction to liberate the
separated and purified biomacromolecule.
In the prior art, removal of particulates by
filtration of liquid streams has been accomplished by
applying one or a combination of the following
25 filtration mechanisms, and liquid filter cartridges are
presently commercially available that operate by each
mechanism.  The present invention utilizes a
modification of these filter cartridges whereby the
filtration layers retain stationary phase particulates
30 in a flowing separation system.
i)    Depth Filtration -- This procedure is one in
which a particulate-containing liquid stream is
confronted by a filter element possessing a
distribution of sized holes or pores and offers the
35 particulates a rather tortuous pathway through the
filtration layer.  In the prior art, particulates were

chiefly removed by adsorption and/or entrapment within
the filtration layer itself. Depth filtration, often
the coarse or first filtration procedure applied to a
system and one designed to remove particulates having a
5 size from hundreds of micrometers (in diameter-largest
dimension) to about 1 micrometer, suffers problems of
incomplete removal of particulates due to ill-defined
pore sizes and steady, rapidly increasing filter
cartridge pressures as the filter becomes loaded.
10        ii)  <u>Surface (Cake) Filtration</u> -- This procedure
is preferred in the present invention and often occurs
subsequent to depth filtration in the treatment of a
liquid stream.  In the prior art, it was generally
conducted using multiple layers of glass or polymeric
15 microfibers which possessed well-defined pore sizes,
and the particulates generally did not penetrate within
the filtration layer but remained trapped on the
upstream surface of the layer.  Particulate sizes down
to about 1.0 micrometer were collected with
20 efficiencies as high as 99.99%.  High flux rates were
readily achievable, and relatively large quantities of
particulates were removed at relatively low system
pressures until the filter was nearly full.  In the
present invention, it may be advantageous to remove or
25 realign the filtered particles on the surface of the
filtration layer by multiple reverses of the liquid
flow; this opportunity does not exist with depth
filters.
          iii) <u>Membrane (Screen or Sieving) Filtration</u> --
30 This filtering mechanism is very similar to surface
filtration, except that precisely defined, very small
pores are present that are capable of removing
virtually all particulates with sizes as low as 0.05
microns.  While providing "absolute" control of
35 particulates remaining in the stream, problems of low

flow rates, low capacity, high pressures, and clogging
also attend this filtration mechanism.

Useful surface filter cartridges in the present
invention include the standard vertical pleated filters
5 of U.S. Patent No. 3,058,594 and, especially preferred,
the horizontal, compound radially pleated filters of
U.S. Patent No. 4,842,739. A horizontal arrangement of
pleats (as shown in FIG. 3) is preferred in the present
invention because the filter cartridges are generally
10 employed vertically, and a greater percentage of
particles is retained within the horizontal pleats when
flow is discontinued and the cartridge stored between
uses. Other filter cartridges such as string wound,
resin bonded, and spray spun depth filters may also be
15 utilized but generally lack the ability to accept as
much particulate as the surface filters while at the
same time maintaining relatively low system pressures.

Standard cylindrical, vertically pleated filter
cartridges are available from Ameteck Inc. (Sheboygan,
20 WI) in a variety of sizes, e.g., 4.8 x 24.8 cm
(diameter x height), 6.7 x 24.8 cm, 6.7 x 50.8 cm, 11.4
x 24.8 cm, and 11.4 x 50.8 cm, with filter element
materials, e.g., cellulose, cellulose-polyester, glass-
cellulose, polyester, polypropylene, and ceramic, and
25 having average nominal pore sizes, e.g., 1, 2, 3, 5,
10, 20, 30, and 50 micrometers. Preferred cylindrical,
compound horizontally radially pleated surface filter
cartridges of all-polypropylene construction can be
purchased from 3M Filtration Products (St. Paul, MN) in
30 a variety of sizes, e.g., 6.4 x 25.0 cm, 6.4 x 50.0 cm,
6.4 x 75.0 cm, and 18.0 x 100.0 cm, and possessing
average nominal pore sizes of 2, 5, 10, and 20
micrometers. Smaller disposable capsule filters that
are useful for smaller scale separations are available
35 from Gelman Sciences, Inc. (Ann Arbor, MI) in a variety
of sizes, e.g., 6.3 x 6.4 cm, 5.8 x 17 cm, and 8.6 x 14

cm, with filter element materials, e.g., polyamide such
as acrylic coated Nylon and polypropylene, and average
nominal pore sizes, e.g., 1, 3, and 5 micrometers.

While the binding (separation step) and elution
5  (isolation step) interactions can be conducted using
filter cartridge housings available from filter
cartridge manufacturers, these housings generally
possess only one set of inlet and outlet ports. As a
consequence, it is difficult to accomplish the highly
10  desirable concentration of the purified
biomacromolecule when the debinding solution is
introduced. A preferred filter cartridge housing of
all-polypropylene construction, available from Ultra
Filter Systems (Brooklyn Center, MN), possesses an
15  additional set of inlet and outlet ports of smaller
size. This smaller set of ports can advantageously be
utilized to accomplish debinding of the
biomacromolecule, generally in a significantly reduced
total volume of solution so that the purified
20  biomacromolecule is obtained in a more concentrated
solution in the process as well.

Preferably, the composition of the filtration
medium of the present invention comprises one or more
nonwoven layers on the upstream surface of which are
25  randomly disposed insoluble stationary phase
particulates. From a mechanical standpoint and with
regard to solvents employed, compositions of filtration
media are not critical when conducting separations
because water is utilized almost exclusively, and
30  essentially all of the above-specified filtration layer
materials generally perform well in water. A preferred
material because of its availability, cost, and
inertness is polypropylene.

Selection of the pore size of the filtration layer
35  depends directly on the size range of the stationary
phase particulates to be retained on the upstream

surface thereof and generally corresponds with the
smallest particulate size.  It has been determined,
however, that even if a portion of the particulates
possesses sizes smaller than the pore size of the
5  filtration layer useful composite filtration media can
be obtained.  Because of the method of preparing the
partially loaded filtration media _vide_ _infra_ these
smaller particulates will pass through the filtering
element in early cycles, in later cycles as a bed of
10  particulates accumulates the device takes on the nature
of a depth filter, and these smaller particles can also
be removed and utilized in the invention.  In the
interests of time efficiency and utilizing the filter
cartridge in the preferred surface filtration mode,
15  however, it is preferable to utilize a surface filter
cartridge unit wherein at least 95% of the stationary
phase particulates are removed in the first pass
through the filter.  Generally a filter cartridge rated
nominally at an average of 1-10 micrometers meets these
20  criteria and provides an efficient filtering element
for the particulates utilized in the invention and also
is capable of delivering relatively high flux rates at
low filter cartridge pressures.  Filtration layers with
average pore sizes less than 1.0 micrometer such as
25  porous, nonfibrous membranes are not generally useful
because they are susceptible to plugging, not only from
adventitious particles that may be present but even by
suspended biological material which is often
encountered in highly concentrated biological solution
30  mixtures.
    For purposes of this invention, stationary phase
particulates bind or strongly associate with the
biomacromolecules of interest in solution mixtures by
one or a combination of the following interactions:
35  adsorption, ion exchange, hydrophobic association, and
affinity binding.  The sizes of the stationary phase

particulates useful in the invention can range from a
distribution in which a small portion, e.g., less than
5%, are submicrometer (largest diameter) to as large as
several millimeters depending on the nature of the
5  filter cartridge employed.  Discussion and caveats
relating to the lower size limit of particulates have
already been given in relation to selection of a proper
pore size filtering layer.  The upper size limit of the
particulates will depend on the particular kind of
10 filter cartridge and is ultimately determined by the
separation of pleat or fold tips.  Particulate sizes
are required to be less than the distance between pleat
or fold inlet tips so that penetration within and on
the upstream surface of the pleats or folds of the
15 filtering element can occur.  As a matter of working
practicality, experience has shown that in order to
prevent aggregates of particles from exceeding the
pleat/fold tip distance, a significantly smaller
particle size, i.e., approximately 1/5 or less of the
20 pleat/fold tip distance, preferably is utilized.  An
important attribute of the present invention is that a
greater quantity of the selected biomacromolecule can
generally be separated by utilizing a particulate
support possessing a relatively high surface area.
25 Preferably, the surface area is at least 10 $m^2/g$, more
preferably at least 50 $m^2/g$, and most preferably at
least 100 $m^2/g$, and even up to 5000 $m^2/g$ (as determined
by gas adsorption measurements).  Particle sizes of
stationary phase materials are preferably in the range
30 of submicrometer to 400 micrometers, more preferably 1-
200 micrometers, and most preferably 10-100 micrometers
in diameter.

Various interactions between solutes and
stationary phase particulates can involve relatively
35 weak attractive forces such as dipole-dipole, ion-
dipole, and ion-ion interactions.  What makes

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biomacromolecules having a molecular weight of at least
500 efficiently bound in the present invention is that
several of these interactions occur over the relatively
large area of contact between biomacromolecule and
5 stationary phase, resulting in a net strong attractive
force.

Adsorption chromatography utilizes the binding
association of polar groups on a stationary phase and
the wide diversity of polar groups on
10 biomacromolecules. These binding associations are
generally of the form of dipole-dipole and ion-dipole
interactions. The binding or separation phase of the
purification operation is usually conducted from an
aqueous buffered solvent of relatively low ionic
15 strength so that the above-mentioned binding
associations between stationary phase and
biomacromolecule solute can be maximized to effect
binding. After washing with a buffered aqueous
solution of low ionic strength, the eluting solution
20 commonly employed contains a relatively large amount of
dissolved salts and a concomitant high ionic strength
so that interactions between the stationary phase and
the dissolved salts will displace the biomacromolecule
from the stationary phase, and the biomacromolecule
25 will re-dissolve and can be recovered in purer form
from the separation system.

Preferred adsorption stationary phase particulates
include hydroxylapatite (available from BioRad
Laboratories, Richmond, CA), alumina (available from EM
30 Separations, Gibbstown, NJ), silica gel (also available
from EM Separations), and zirconia (disclosed in U.S.
Patent No. 5,015,373).

Ion exchange chromatography takes advantage of the
fact that many biomacromolecules are ionically charged.
35 Furthermore, many of these ionically charged groups,
e.g., protonated amine and carboxylate, can be rendered

neutral and uncharged by a change in pH.  This provides
a sensitive and very powerful technique for separation
of biomacromolecules based on their isoelectric points,
often indicated by a pI value which is the pH at which
5   charge neutrality exists or the number of negatively
charged groups and positively charged groups within a
structure is the same.  If the pH is maintained above
pI, then an anion exchange resin can be used to bind
the biomacromolecule; conversely, if the pH is lower
10  than pI, a cation exchange resin can effect binding and
removal of the biomacromolecule from the solution
mixture.  With this technique even small differences in
accessible or surface charges on biomacromolecules can
result in effective separations.  After washing the
15  insolubilized biomacromolecule:stationary phase
particulate product, elution of the bound
biomacromolecule from an ion exchanging stationary
phase particulate is generally conducted by introducing
a relatively high concentration of a salt solution
20  whose corresponding ions will exchange with and
displace the biomacromolecule from the stationary phase
particulate.

    Useful ion exchange stationary phase particulates
are available from several vendors including Pharmacia
25  LKB Biotechnology, Inc. (Piscataway, NJ), Toso Haas
(Philadelphia, PA), EM Separations (Gibbstown, NJ), and
Biosepra, Inc. (Marlborough, MA).  Useful anion
exchanging resins feature agarose, dextran, and
cellulose polymers that have been modified to contain
30  diethylaminoethyl and quaternary ammonium groups.
Cation exchanging resins feature the same base polymers
but possessing carboxylate and sulfonate groups.

    Hydrophobic interaction chromatography utilizes
the principle of "like dissolves like" and the
35  hydrophobicity of many biomacromolecules.
Intercalation of hydrophobic portions of a

biomacromolecule into hydrophobic pockets of a
stationary phase particulate results in a binding
association and separation of the biomacromolecule from
the solution mixture.  The procedure is commonly
5 conducted by binding from an aqueous solution of
relatively high ionic strength.  In this fashion the
biomacromolecule is somewhat precariously soluble to
begin with by being almost "salted out" of solution and
will readily bind to a hydrophobic solid support.
10 Elution is commonly conducted by employing an aqueous
solution of reduced ionic strength (and increased
solvent efficiency for the biomacromolecule);
alternatively, organic solvents such as acetone,
acetonitrile, ethanol, methanol, and N,N-
15 dimethylformamide in amounts up to 50 weight percent
may be employed with water as co-solvent to remove the
biomacromolecule from the insoluble complex with the
stationary phase particulate.

        Useful hydrophobic interaction stationary phase
20 particulates can be purchased from Pharmacia, among
other vendors, and feature agarose base supports.  Any
of the hydrophobic interaction stationary phase
particulates can be modified by inclusion of butyl,
octyl, and phenyl groups.

25      Affinity chromatography operates generally by
covalently binding a ligand or biospecific effector to
a stationary phase particulate.  This ligand or
effector is chosen because of its ability to interact
with a biomacromolecule by a "lock and key"
30 relationship.  If a protein, for example, is the
biomacromolecule whose separation is desired, the
covalently bound ligand or effector is often a
substrate or inhibitor ("key" molecule) which binds
strongly to the active site (the "lock") of the
35 protein.  The high selectivity of this process allows
for one step purification of a biomacromolecule from a

complex mixture.  Although elution is often
accomplished by a simple change in pH, debinding
solutions and techniques are specific for each
biomacromolecule-ligand pair, and specific instructions
5 can be obtained from the manufacturer.

       Useful affinity chromatography stationary phase
particulates are available from Pharmacia, Toso Haas,
EM Separations, Biosepra, Inc., and 3M Bioapplications
(St. Paul, MN).  A variety of base particulate supports
10 including agarose, cellulose, and vinyl polymers is
available, for example, from Pharmacia, Piscataway, NJ,
possessing several ligands (with corresponding
biomacromolecule affinities) including:  arginine and
benzamidine (serine proteases), Cibacron Blue (enzymes
15 requiring adenyl-containing cofactors, albumin,
coagulation factors, interferon), calmodulin (ATPases,
protein kinases, phosphodiesterases, neuro-
transmitters), gelatin (fibronectins), glutathione (S-
transferases, glutathione-dependent proteins, fusion
20 proteins), heparin (growth factors, coagulation
proteins, steroid receptors, restriction endonucleases,
lipoproteins, lipases), Proteins A and G (IgG and
subclasses), L-lysine (plasminogen, plasminogen
activator, ribosomal RNA), procion red ($NADP^+$ dependent
25 enzymes, carboxypeptidase G), concanavalin A and
lectins (glycoproteins, membrane proteins, glycolipids,
polysaccharides), and DNA (DNA polymerase, RNA
polymerase, T-4 polynucleotide kinase, exonucleases).

       Having thus described the filter cartridges,
30 filter housings, and stationary phase particulates, the
process by which the separation systems are prepared
will now be detailed.  The process involves the steps
of:

       i)    providing a closed loop assembly comprising a
35 filter cartridge comprising a composite filter medium
of the invention contained in a housing, a reservoir

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containing a slurry of the insoluble stationary phase
particulates in a liquid, and a pump and associated
tubing capable of delivering a flux rate of at least
0.01 cm/minute;

5         ii)   pumping the slurry through the filter
cartridge in a recycling mode until the desired amount
of stationary phase particulates has been loaded;
preferably the filter cartridge pressure is less than
about 0.15 MPa, more preferably less than 0.10 MPa, and
10  most preferably less than 0.05 MPa; and

          iii)  introducing a biological solution mixture
which comprises at least one biomacromolecular solute
into the reservoir so that it can undergo circulation
in the separation filter assembly to effect separation
15  of the biomacromolecule.

          Pumps useful in the invention provide flux rates
through the filter cartridge in excess of 0.01
cm/minute, preferably in excess of 0.10 cm/minute, and
more preferably in excess of 0.30 cm/minute.  The pumps
20  and associated gasketing and tubing/piping through
which at least one of the slurry and solution mixture
comprising more than one biomacromolecule solute flow
preferably are relatively chemically unaffected by the
solution.  Preferred pumps include peristaltic,
25  diaphragm, gear, and centrifugally driven pumps in
which the actual pump components contacting the
solution are constructed of stainless steel or
polytetrafluoroethylene (PTFE).  Most types of rubber
or plastic tubing/piping are suitable for packings and
30  separations conducted in aqueous media, but if aqueous
mixtures of organic solvents are employed,
polypropylene, polyethylene, PTFE, stainless steel, and
glass tubing preferably are employed.  Preferred
gasketing materials to interface the connection of the
35  filter cartridges to filter housings and with the rest

of the separation system include PTFE and
polypropylene.

In contrast to conventional "dry" packing
manufacturing techniques, "wet" packing the
5 particulates onto the filtration layer by use of a
liquid carrier assures that the particulates are
located in regions of the filtering element which are
subsequently accessible to solution mixtures. The
particulates are randomly located on the filtration
10 layer in the sense that their positions are not pre-
selected, although the flow of the liquid carrier may
influence the ultimate location of particulates. In
packing the filter cartridge by the above process it is
desirable to employ fairly dilute concentrations of the
15 particulates in the liquid during each packing session
in order to achieve relatively uniform partial loading
of the filtering element. The particulates can be
added to the reservoir in a portionwise fashion (either
without solvent if suitably dense and water-wettable or
20 pre-slurried), with visual clarification of the
reservoir contents occurring between each portion. The
flux rate of the packing operation preferably is at
least 0.01 cm/minute, more preferably at least 0.10
cm/minute, and most preferably at least 0.30 cm/minute.
25 In addition to separating desired biomacromolecules
efficiently with regard to quantity and time during the
separation phase, relatively high flux rates are
desirable during the particle loading phase especially
with the preferred compound radially pleated filter
30 cartridges so that the particulates can better permeate
the folds of the pleated filter element, thus accessing
more of the filter element and facilitating high
loading. The liquid employed to slurry the stationary
phase particulates generally is the solvent of the
35 solution mixture and is generally water, preferably
buffered water. With hydrophobic interaction

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stationary phase particulates it may be necessary,
especially in the elution step, to utilize organic
liquids, in combination with water.  Useful organic
liquids include methanol, ethanol, isopropanol,
5  acetonitrile, and N,N-dimethylformamide in amounts up
to 50 weight percent.

        The particulates are loaded into the reservoir and
ultimately onto the upstream surface of the filter
element until the filter cartridge pressure reaches not
10  more than 0.15 MPa, preferably not more than 0.10 MPa,
and more preferably not more than 0.05 MPa.  A
practical filter cartridge pressure limit for a fully
loaded preferred compound radially pleated filter
cartridge is about 0.25 MPa.  As a general rule of
15  application of filter cartridges, when filter cartridge
pressures in excess of about 0.05 MPa are attained,
subsequent loading of additional particulates results
in increasingly higher filter cartridge pressures.
Especially with the lower recommended filter cartridge
20  pressures, however, flux rates of solutions passing
through the filter cartridges remain high and in the
range desirable for the purposes of this invention.  In
this fashion, the unit can still respond to
adventitious particulates that are likely to be
25  encountered during subsequent separation and handling
operations.  By reserving some particulate loading
capacity for actual operation, shut downs due to filter
plugging are averted and filter cartridge lifetimes can
be extended.
30      The stationary phase particulate loaded filter
cartridge is now ready to be utilized as a separation
filter assembly to separate a biomacromolecule from a
solution mixture passed through.  The separation filter
assembly and cartridge are schematically illustrated in
35  FIGS. 1-5.

After loading the particulates to provide the
composite filtration media, the inlet and outlet tubing
ends are removed from the reservoir (or left attached
if the packing reservoir will also function as
5    separation reservoir) and are attached to a reservoir
containing a biological solution mixture.  Biological
solution mixtures can comprise more than one bio-
macromolecule solute which is (or at one time was) a
component or product of a cell.  The desired
10   biomacromolecule can be a product that is excreted from
a cell or an intracellular component obtained after
lysis of the cell's outer membrane or wall.  Useful
biological solution mixtures include fermentation
media, cell lysates, and body fluids such as blood and
15   blood components, ascitic fluids, and urine.
     It is normally desired to obtain the greatest
quantity of purified biomacromolecule in the shortest
period of time.  The velocity with which a solution
mixture is passed through the composite filtration
20   medium, i.e., the flux rate, and recycled has been
determined to be an important criterion for performance
in the present invention.  One very important factor is
that the present invention separation filter assemblies
permit larger volumes of solution mixtures to be
25   processed in a given time primarily because of low
pressure operation.  Other factors which may contribute
to the high efficiencies of the present invention
separation filter assemblies are:  1) the ability to
utilize smaller stationary phase particulates
30   possessing relatively high surface areas and reduced
diffusional limitations compared to larger particles
utilized in packed columns; 2) better shear mixing of
the biomacromolecule solutes and the stationary phase
particulates at higher flux rates; and 3) access to a
35   greater number of particulates contained deeply within
pleats or folds of the filtering element at higher flux

rates. A flux rate of solution mixture passage of at
least 0.01 cm/minute is preferred, more preferably at
least 0.10 cm/minute, and most preferably at least 0.30
cm/minute.

5       The filter elements of the present invention find
utility in a variety of biological separations
involving proteins, carbohydrates, lipids, nucleic
acids, and other biological materials. Separated and
purified macromolecules are useful therapeutic and
10  diagnostic agents.

        Objects and advantages of this invention are
further illustrated by the following examples, but the
particular materials and amounts thereof recited in
these examples, as well as other conditions and
15  details, should not be construed to unduly limit this
invention.


Example 1

        This example teaches use of a separation filter
20  assembly of the invention in separating a
biomacromolecule from a solution mixture using an
adsorption interaction.

        A separation system shown in FIG. 6 was
constructed using a 6 liter beaker as packing and
25  separation reservoir; a separation filter assembly
consisting of a 2 $\mu$m nominal pore size, horizontal,
compound radially pleated filter cartridge of all-
polypropylene construction and possessing a filtration
layer with a frontal surface area of 0.84 m$^2$ (Model
30  313 B, available from 3M Filtration Products, St. Paul,
MN) and a Lexan filter housing (Model PSCL, available
from Ametek, Sheboygan, WI); a Masterflex peristaltic
pump (Model 7549-30, available from Cole-Parmer
Instrument Co., Chicago, IL); and Masterflex Pharmed
35  Norprene tubing (Model No. 6485-73, also available from
Cole-Parmer).

A buffer solution (4 L of 0.005 M sodium
dihydrogen phosphate and 0.005 M disodium hydrogen
phosphate at pH 6.5) was placed in the reservoir, and
hydroxylapatite (Biogel HTP™, available from Bio Rad
5 Laboratories, Richmond, CA) (60 grams in 5 gram
increments) was loaded onto the upstream surface of the
filter cartridge using a flow rate of 3800 mL/minute
(flux rate = 0.45 cm/minute). A pressure gauge located
on the upstream side of the separation filter assembly
10 recorded an increase in pressure of only 0.02 MPa
during the loading operation.

Separation Phase:
        To the buffer solution in the reservoir were added
15 3.25 grams of hemoglobin (bovine, available from Sigma
Chemical Company, St. Louis, MO) pre-dissolved in 500
mL of buffer, and the pump was started at a rate of
3800 mL/minute. Within 5 minutes, UV analysis
(absorbance at 408 nm) showed that 95% of the
20 hemoglobin had been removed from the reservoir
solution.

Isolation Phase:
        The contents of the reservoir were discarded, and
25 the separation filter assembly was washed (in one pass)
with 4 L of distilled water. Next, an eluting solution
(0.25 M sodium dihydrogen phosphate and 0.25 M disodium
hydrogen phosphate at pH 6.5) (3300 mL) was placed in
the reservoir. Pumping was begun at 3800 mL/minute and
30 81% of the bound hemoglobin was recovered in 3300 mL of
solution in 3 minutes based on UV absorptions.

Example 2
        This example shows the principle of the use and
35 advantages of a separation filter assembly having

different separation and isolation loops for purposes
of increasing the concentration of a biomacromolecule.

A separation filter assembly shown in FIG. 5 was
utilized and was obtained from Ultrafilter Systems
5  (Brooklyn Center, MN). The unit featured all-
polypropylene construction and a 3M (Model 313 B)
filter cartridge; the separation loop fittings were
1.50 cm in diameter (inside), while the isolation loop
fittings were 0.50 cm in diameter. Hydroxylapatite (60
10  grams) was loaded onto the filter cartridge as
described in Example 1 using the same buffer solution,
quantities, and flux rate.


Separation Phase:
15       Using the 1.50 cm fittings and a general
arrangement depicted in FIG. 6 with 0.90 cm (inside
diameter) tubing, hemoglobin (2.00 grams) was bound to
the hydroxylapatite from a total system volume of 6100
mL using a flux rate of 0.45 cm/minute. After 30
20  minutes, UV analysis revealed that 95.8% of the
hemoglobin had been removed from the reservoir
solution. The unit was then washed (in one pass) with
distilled water (4L).


25  Isolation and Concentration Phases:
The distilled water remaining in the separation
filter assembly was drained through the center outlet
port (0.50 cm fitting). Then, using the smaller set of
fittings as the isolation loop and a smaller Masterflex
30  pump (Model 7021-36) equipped with 0.50 cm (inside
diameter) tubing, an eluting solution as disclosed in
Example 1 (650 mL) was recirculation pumped at 500
mL/minute through the cartridge for 3 minutes; UV
analysis of the solution when subsequently pumped and
35  drained from the assembly showed 51% recovery of
hemoglobin. Another 650 mL of debinding solution

similarly employed removed an additional 19%.
Therefore, 70% of the hemoglobin had been isolated and
collected in more concentrated form in 1300 mL of
solution, compared with the original volume of 6100 mL.

5

Example 3

This example teaches use of an ion exchange
stationary phase particulate for separating a
biomacromolecule from a solution mixture containing
10  more than one biomacromolecule.  At pH 8.0, cytochrome
C (pI = 9.6) (horse heart, available from Sigma) was
positively charged and was not bound by the positively
charged, protonated diethylaminoethyl (DEAE)-functional
stationary phase particulates.  Bovine serum albumin
15  (BSA; pI = 5.5) (available from Miles Diagnostics,
Kankakee, IL), on the other hand, was negatively
charged and was bound at pH 8.0.

The separation system of FIG. 6 was utilized in
which the separation filter assembly consisted of a
20  filter cartridge (Model 313 B, 3M Filtration Products)
and a filter cartridge housing depicted in FIG. 5
(Ultra Filter Systems).  The reservoir contained 4 L of
sodium citrate buffer solution (pH 6.0), and DEAE
Sepharose Fast Flow™ (110-160 µeq./mL, available from
25  Sigma) (500 mL) was portionwise loaded over the course
of 30 minutes while pumping at 3800 mL/minute (flux
rate = 0.45 cm/minute).  A corresponding filter
cartridge pressure of about 0.01 MPa was observed over
the packing period.  A solution (25 mM; 3L) of EPPS (N-
30  [2-hydroxyethyl]piperazine-N'-[3-propanesulfonic acid])
buffer (pH 8.0) (Sigma Chemical Co., St. Louis, MO) was
then pumped (one pass) through the separation filter
assembly to replace the packing citrate buffer.

35

Separation Phase:

       The solution in the reservoir was replaced with
3850 mL of 25 mM EPPS buffer (pH 8.0). A solution
mixture consisting of 100 mg of cytrochrome C (horse
 5 heart, available from Sigma), 500 mg of BSA and 40 mL
of EPPS buffer was added to the reservoir bringing the
total separation system volume to 5350 mL (3850 mL + 40
mL + 1460 mL (volume in separation filter assembly and
tubing)). The UV spectrum of the solution in the
10 reservoir was recorded, with special attention being
paid to the absorbance at 280 nm which was 0.199
(chiefly due to BSA); cytochrome C exhibited a major
peak at 408 nm (A = 0.231). The pump was engaged at
3000 mL/minute (flux rate = 0.36 cm/minute), and
15 aliquots were removed from the reservoir every two
minutes. Approximately 20 minutes of recirculation
pumping were required until the absorbance at 280 nm
diminished no further; the absorbance at 408 nm
remained essentially unchanged in all of the aliquots.
20 Thus, cytochrome C remained in solution while the BSA
became ionically bound onto the stationary phase
particulates in the separation filter assembly.


       Isolation Phase:

25     First the separation filter assembly was washed
(one pass) with 3 L of 25 mM EPPS. Then, using the
same loop, i.e., the 1.5 cm fittings, 1 M NaCl was
pumped (flux rate = 0.36 cm/minute) through the unit
(one pass). UV analysis of the eluant revealed that
30 67% of the purified BSA was obtained in the first 3 L.


       Example 4

       This example teaches the separation of BSA and
cytochrome C using hydrophobic interaction binding.
35     Macro Prep™ t-Butyl HIC™ Support (available from
Bio-Rad Laboratories, Hercules, CA) which features a

hydrocarbon polymer backbone and pendent t-butyl groups
was utilized as the hydrophobic interaction stationary
phase particulate.  The support consisted of beads
possessing a nominal bead diameter of 50 micrometers;
5  the material was available as a slurry in 20 weight
percent ethanol-water, and the dry polymer content was
18.6% by weight. This material (40 mL of the slurry;
7.68 grams) was added in 5 mL portions to a reservoir
containing 2M ammonium sulfate.  The particulates were
10  loaded by the procedure of Example 1 onto the upstream
surface of a polypropylene filter cartridge (3M; Model
313B) employed in a configuration as depicted by the
separation system shown in FIG. 6 using a flow rate of
4926 mL/minute (flux rate = 0.59 cm/minute); total
15  system volume was 6004 mL and the pH was 5.4.

Preliminary UV analyses were conducted on
cytochrome C and in combination with an equal weight of
BSA.  Ratios of absorbances at 280 nm/408nm for
cytochrome C were 0.148/0.453 = 0.327 and in
20  combination with BSA an absorbance ratio of 0.179/0.461
= 0.388.

The separation system was then challenged with 300
mg of cytochrome C and 300 mg of BSA by adding the
protein solids to the reservoir and stirring.  When
25  dissolved, the pump was begun at the above flux rate,
and 1 mL aliquots were withdrawn at various times and
the optical densities at 280 and 408 nm were measured.
The data is shown in Table 1, below.

| Table 1 | | | |
|---|---|---|---|
| Time (minutes) | Optical Density (OD) at 280 nm | OD at 408 nm | 280/408 Ratio |
| 5 | 0.174 | 0.466 | 0.373 |
| 10 | 0.174 | 0.460 | 0.366 |
| 15 | 0.167 | 0.460 | 0.363 |
| 30 | 0.163 | 0.456 | 0.357 |
| 45 | 0.161 | 0.452 | 0.356 |
| 60 | 0.164 | 0.455 | 0.360 |

The data of Table 1 show that the more polar cytochrome C remained in solution while the relatively hydrophobic BSA (near its pI of 5.6) was bound selectively onto the stationary phase particulates of the separation filter assembly.

At this point the assembly can be washed with 2M ammonium sulfate in a one-pass operation to remove all nonspecifically bound biomacromolecules. The BSA can then be isolated by passing an eluting solution consisting of a reduced ionic strength solution such as 0.1 M ammonium sulfate, preferably at the same pH, through the unit.


Example 5

This example teaches the use of a separation filter assembly of the invention to separate specific biomacromolecules from a solution mixture comprising many biomacromolecules using an affinity interaction.

A modified version of the separation system of FIG. 6 was utilized in which the filter cartridge comprised a 2 $\mu$m nominal porosity horizontal, compound radially pleated filter possessing a surface area of 0.37 m$^2$ (available from 3M Filtration Products, St.

Paul, MN). Also, an in-line UV absorbance monitor
(Model UA-5; available from ISCO Instrument Co.,
Lincoln, NE) was incorporated on the downstream side of
the separation filter assembly. A buffer solution (4
5  L) consisting of 0.15 M sodium chloride, 0.003 M sodium
dihydrogen phosphate, and 0.017 M disodium hydrogen
phosphate (pH = 7.4) was added to the reservoir and
recirculated through the system using a flow rate of
4000 mL/min. (flux rate = 1.08 cm/min). The filter
10 assembly was carefully vented to remove all trapped
air. EMPHAZE™ Biosupport Media derivatized with
recombinant Protein A (80 mL; available from 3M
Bioapplications, St. Paul, MN) was added to the packing
reservoir in 20 mL aliquots and was loaded onto the
15 upstream surface of the filter element using
recirculation pumping at 4000 mL/min. After all the
Protein A-functional stationary phase particulates had
been deposited, the system flow rate was increased to
6000 mL/minute for 2 min. to ensure that the particles
20 were deposited into the pleat folds. System flow rate
was then reduced to 1000 mL/min. and the assembly was
washed in one pass with 6 L of buffer solution. At
this point, system flow was stopped, and the packing
reservoir was replaced by a 4 L polypropylene container
25 equipped with a magnetic stirring bar in order to
perform the separation operation.

Separation Phase:
       The separation reservoir was charged with 2000 mL
30 of buffer solution described immediately above and the
system flow rate was 1000 mL/min. (flux rate = 0.27
cm/min.). Filtered cryo-poor human blood plasma (350
mL; obtained from the American Red Cross St. Paul
Regional Blood Center, St. Paul, MN) was added to the
35 reservoir, and recirculation was continued for 30 min.

The system was then washed with 4 L of fresh buffer
solution in one pass.

Isolation Phase:

5        Protein captured in the separation filter assembly
was then eluted using a solution (2 L) consisting of
0.1M glycine and 2 volume % acetic acid (pH = 2.2).
This solution was recirculation pumped through the
assembly for 15 min. at 1000 mL/min.  Eluted protein
10  was then isolated by one pass pumping the system and
adding fresh buffer to the reservoir until a total of
4000 mL was collected.
         The eluant was then analyzed to determine the
identity, purity, and concentration of separated
15  proteins.  Analysis of chemically reduced samples of
the eluate with SDS gel electrophoresis (Pharmacia
PhastGel™, 8-25% gradient gel with silver stain) (FIG.
7) indicated that the eluted protein was composed
almost entirely of immunoglobulins by comparison with
20  an authentic commercial sample.  Measurement of the
optical density of the recovered eluate at 280 nm
indicated that the concentration of protein was
approximately 0.27 grams/liter.  Therefore,
approximately 1.08 grams of pure human immunoglobulins
25  were recovered from the 350 mL of blood plasma.  This
corresponds to a yield of about 31% if the
immunoglobulin concentration in cryo-poor human plasma
is assumed to be 10 grams/liter.


30        Various modifications and alterations of this
invention will become apparent to those skilled in the
art without departing from the scope and spirit of this
invention, and it should be understood that this
invention is not to be unduly limited to the
35  illustrative embodiments set forth herein.

We Claim:

1.   A method of separating a biomacromolecule
comprising the steps of
5        a) providing a separation system containing a dead
end filter cartridge comprising a composite filter
medium on the upstream surface of which are located
stationary phase particulates capable of binding with a
biomacromolecule, a reservoir containing a solution
10  mixture comprising at least one biomacromolecule as
solute, and a pump and associated tubing to form a
closed loop assembly,
         b) recirculation pumping the solution mixture
through the filter cartridge so as to bind said at
15  least one biomacromolecule to the stationary phase
particulate so as to form a biomacromolecule:stationary
phase particulate product,
         c)   optionally, washing the biomacromolecule:
stationary phase particulate product with liquid to
20  remove unwanted biomacromolecules and other solutes not
bound to the stationary phase particulates, and
         d)   optionally, pumping an eluting solution
through the closed loop assembly which is capable of
reversing the biomacromolecule:stationary phase
25  particulate product binding interaction so as to
liberate the biomacromolecule.

2.   The method according to claim 1 wherein said
stationary phase particulates are selected from the
30  group of particulates capable of binding by adsorption,
ion exchange, hydrophobic binding, and affinity
binding.

3.   The method according to claims 1 or 2 wherein
35  said pumping of said solution mixture through the
filter cartridge is at a flux rate of at least 0.01

cm/minute, at a filter cartridge pressure of at most
0.25 mPa.

5    4.    The method according to any of claims 1 to 3
wherein said biomacromolecule is selected from the
group consisting of a protein, carbohydrate, lipid, and
nucleic acid.

     5.    The method according to any of claims 1 to 4
10   wherein said composite filter medium comprises a woven
or nonwoven porous material.

     6.    The method according to any of claims 1 to 5
wherein said filter medium material is selected from
15   the group consisting of cellulose, glass, polyolefin,
polyester, polyamide, and ceramic.

     7.    The method according to any of claims 1 to 6
wherein said filter medium material is polypropylene.
20

     8.    The method according to any of claims 2 to 7
wherein said adsorption stationary phase particulates
are selected from the group selected from
hydroxylapatite, alumina, silica gel, and zirconia.
25

     9.    The method according to any of claims 1 to 8
wherein said particulates are affinity chromatography
stationary phase particulates.

30   10.    The method according to any of claims 1 to 9
wherein said biomacromolecule containing solution
mixture comprises a mixture selected from the group
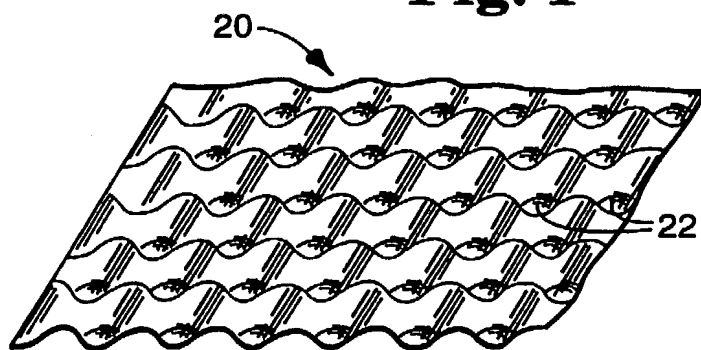consisting of fermentation media, cell lysates, and
body fluids.
35

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11.    A biomacromolecule/particulate product
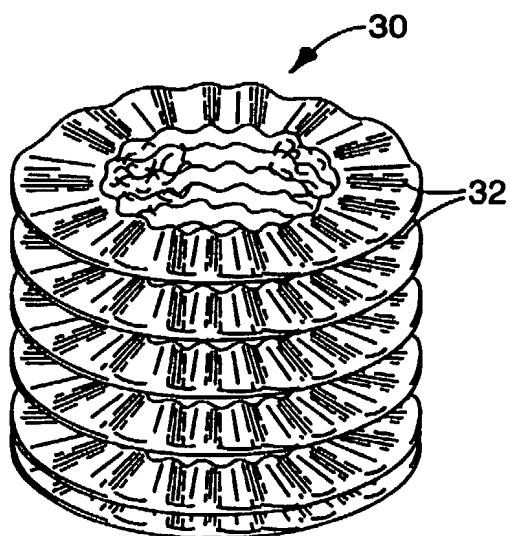provided according to the method of any of claims 1
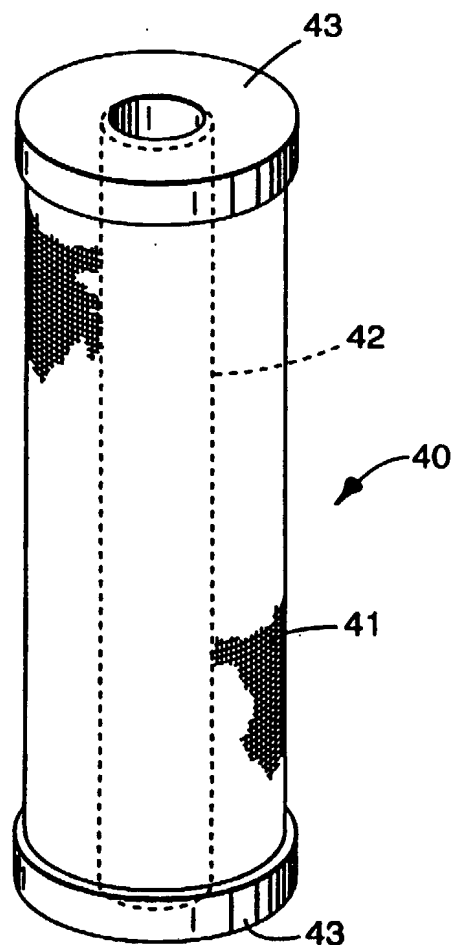to 10.
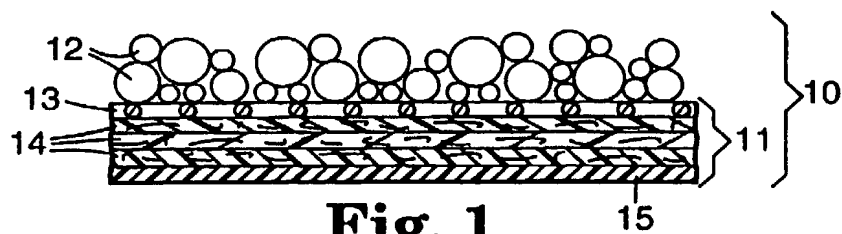
**Fig. 1**



**Fig. 2**



**Fig. 3**
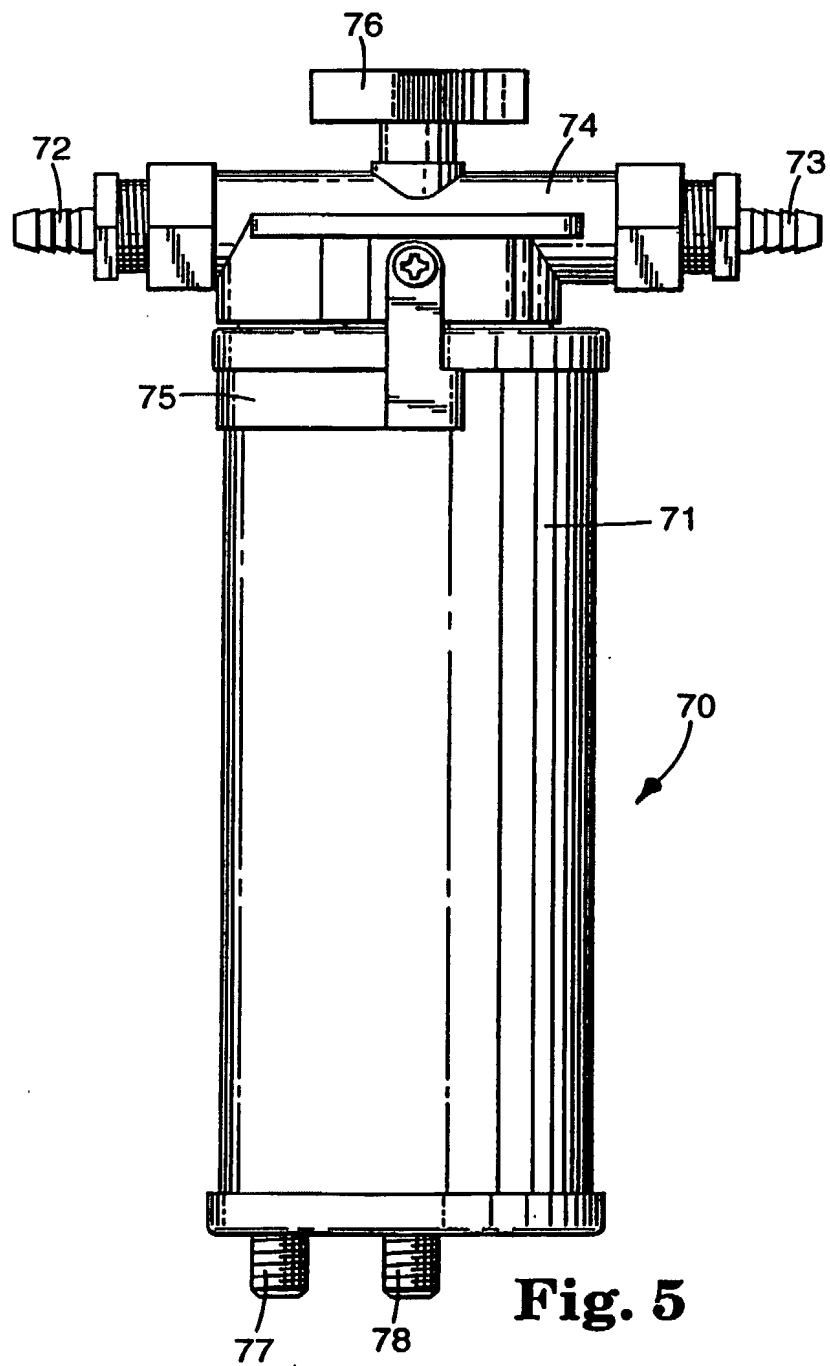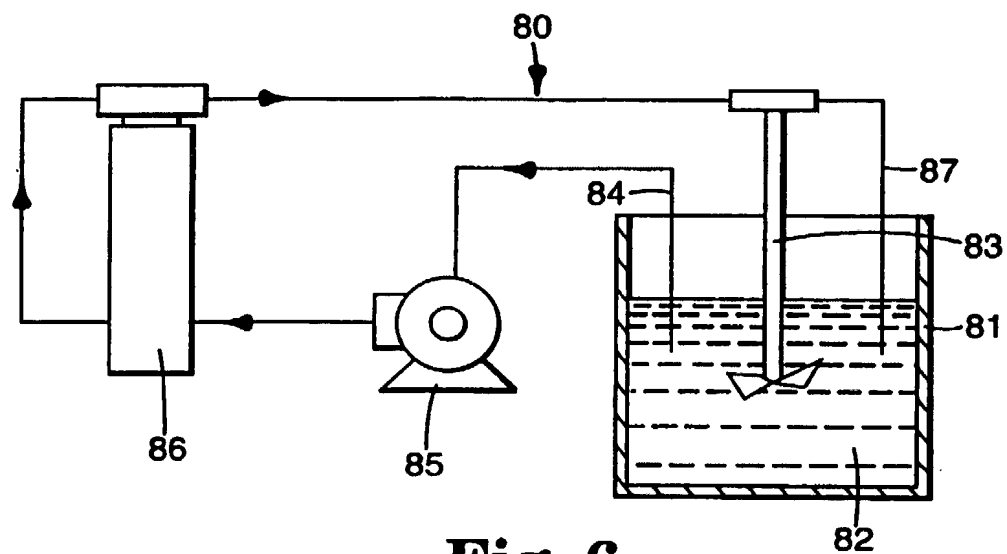


**Fig. 4**

**Fig. 5**

**Fig. 6**
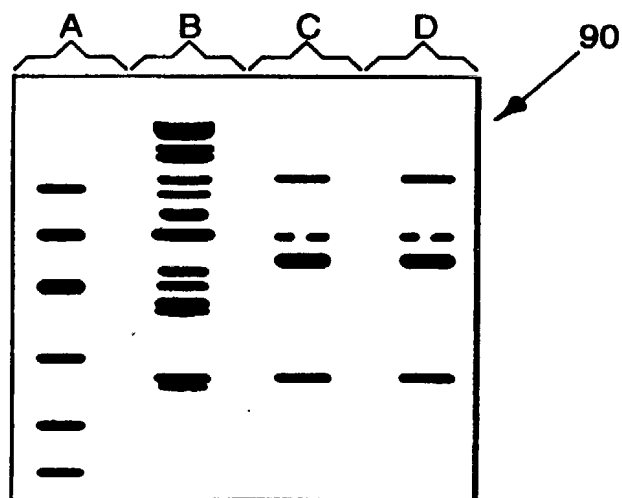


**Fig. 7**

# INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6    C07K1/14        B01D15/08        B01D39/00        B01J20/28

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6    C07K    B01D    B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | 'Pierce 1989 Handbook & General Catalog' 1989 , PIERCE<br>* see page 62, number 20055 * | 1-11 |
| X | 'Supelco Chromatography Products (Catalog)' 1994 , SUPELCO<br>* see p. 300 and 304: feature "P" (mesh support) * | 1-11 |
| A | WO,A,82 00774 (AMF INC.) 18 March 1982 cited in the application<br>* p. 1-22 * | 1-11 |

[ ] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 26 May 1995 | 2 1 -06- 1995 |

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| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Hermann, R |

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
|---|---|---|---|---|
| WO-A-8200774 | 18-03-82 | US-A- | 4384957 | 24-05-83 |
| | | AU-B- | 543079 | 28-03-85 |
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| | | EP-A,B | 0047617 | 17-03-82 |
| | | US-A- | 4512897 | 23-04-85 |